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SUPPLEMENTARY INFORMATION

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Supporting Online Material for

Global prevalence of symbiotic bacterial methane oxidation in peat moss ecosystems

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Supplementary Materials and Methods

Sample collection

Intact *Sphagnum* mosses were collected from nine different peat lands around the world (Supplementary Table S1). Whole and alive, chlorophyll containing mosses were used. When possible, mosses from pool, lawn and hummock were sampled.

Methane oxidizing activity tests

Intact *Sphagnum* mosses were thoroughly washed 3 times with sterile demineralised water, and incubated in 120 ml serum bottles, sealed with airtight, butyl rubber stoppers. To each bottle 1 ml pure methane was added and methane concentrations were monitored daily. Incubation was performed at 4, 10, 15 and 20°C in the dark. When available, peat bog water was tested for methane oxidation. Methane was measured on a HP 5890 gas chromatograph equipped with a flame ionization detector and a Porapak Q column (100/120 mesh). Acetylene addition, irreversibly stopping methane oxidation, was performed with samples from the Mariapeel and Hatertse Vennen.

Methane emission measurements

9 Peat cores (diameter: 15 cm, length 40 cm) were taken from very wet *Sphagnum cuspidatum* dominated hollows from two different peatlands (Tuspeel (51°19'92"N; 5°88'29"E) en Haaksbergerveen (52°13'01"N; 6°77'27"E), The Netherlands). Approximately the upper 10 cm of the cores consisted of living *Sphagnum* moss. The peat cores were filled with water till the top and methane emissions were measured at 18 °C. After a month of pre-incubation at ambient light levels and 18 °C, methane emission rates were measured by means of a funnel (diameter 12.5 cm) closed with a rubber stopper, which was placed upside-down onto the water surface of the column. Accumulation of CH_4 under the funnel was measured every 2 h over a period of 12 h by

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carefully taking air samples with syringes. As CH_4 concentration showed a linear increase, methane emission could be calculated by simple linear regression. Afterwards the upper 6 cm of the living parts of the *Sphagnum* mosses were carefully cut away and two days later methane emissions were measured again.

$^{13}CH_4$ and $^{13}CO_2$ incorporation into lipids

Submerged labelling experiments were performed as described previously[3] on *S. cuspidatum* from the UK, *S. magellanicum* from Argentina and *S. magellanicum* from Canada. Briefly, intact *Sphagnum* mosses were thoroughly washed and incubated in 250 ml serum bottles, submerged in 150 ml medium resembling bog water, after which 99% ^{13}C-labelled methane was added to a final concentration of 200 µM. In parallel $^{13}CO_2$ experiments were performed with the Canadian mosses in the same way as described above, with non labelled methane and the addition of 4.2 mg of labelled sodium bicarbonate. Phosphoric acid was used to adjust the pH to 4.5 in all experiments. The bottles were shaken at 150 rpm at ambient conditions, and sacrificed for lipid analyses at two points in time. Control experiments, where *Sphagnum*, was incubated in air, were performed as described in the previous section, except with 99% ^{13}C labelled methane. Measurements of CO_2 and CH_4 were performed on a GC/MS (5975C, Agilent technologies).

Total lipid extracts of freeze-dried *Sphagnum* species were obtained with an Accelerated Solvent Extractor (Dionex), using a mixture of dichloromethane (DCM):methanol (MeOH) (9:1, v/v). An aliquot of the total extract was methylated with borontrifluoride-methanol (BF_3/MeOH) at 60 °C for 20 min, after which it was separated into three increasingly polar fractions over an activated Al_2O_3 column using hexane:DCM (9:1 v/v), DCM and DCM:MeOH (1:1 v/v), respectively. The DCM:MeOH (1:1 v/v) fraction was subsequently silylated with a 1:1 mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA) (1% TMS) and pyridine at 60 °C for 20 min. An aliquot of the total extract was treated with H_5IO_6 and $NaBH_4$, as described by Rohmer et al. (1984)[10], to cleave the C-C bonds of vicinal polyols, and subsequently silylated.

Fractions were analyzed on a gas chromatograph (HP 6890) equipped with a flame ionization detector (FID) set at constant pressure (100 kPa). A fused silica column (30 m x 0.32 mm i.d., film thickness 0.1 µm) coated with CP Sil-5CB was used with helium as a carrier gas. Extracts were injected on-column at 70 °C. The temperature increased with 20 °C/min to 130 °C and 4 °C/min to 320 °C, followed by an isothermal hold for 20 min. Components were identified using a gas chromatograph–mass spectrometer (Thermo Trace GC Ultra).

Compound specific $\delta^{13}C$ values were determined by Isotope-Ratio-Monitoring Gas Chromatography Mass Spectrometry (GC-IRMS, ThermoFinnigan Delta-Plus XP). Carbon isotopic values are reported in the delta notation relative to the VPDB standard. The $\delta^{13}C$ values of the alcohols were corrected for the attached TMS groups derived from BSTFA, which were determined offline.

The extent of methane-derived CO_2 incorporation was estimated by comparing label uptake in phytosterol (measured value minus the natural isotope abundance) to the enrichment the $^{13}CO_2$ treated sample, through time. The isotope ratios of the gases were monitored through time. The experiment in which *Sphagnum* was incubated with $^{13}CH_4$ showed gradual build up of $^{13}CO_2$, up to 16%, in the headspace. This was

corrected for using the effect calculated based on the parallel $^{13}CO_2$ labelling experiment. Differences in label in CO_2 and CH_4, changes therein over time, and differences in duration of experiments, were accounted for. The calculation was as follows, where $\Delta^{13}C$ stands for the enrichment in ^{13}C in plant sterol relative to the natural isotopic abundance, in atomic percentages (corrected for % ^{13}C in the substrate and duration of the experiment): $\Delta^{13}C_{CH4} + \Delta^{13}C_{CO2} = 100\%$. The $\Delta^{13}C_{CH4}$ was obtained when the measured $\Delta^{13}C_{CH4}$ was subtracted by the amount of ^{13}C incorporated as a result of build up of labeled CO_2 in the headspace derived from methanotrophic respiration: $\Delta^{13}C_{CH4} = \Delta^{13}C_{CH4\ measured} - \Delta^{13}C_{CH4\text{-}derived\ CO2}$ in which $\Delta^{13}C_{CH4\text{-}derived\ CO2} = (^{13}CO_{2\ headspace\ 13CH4\ exp} / ^{13}CO_{2\ headspace\ 13CO2\ exp})^* \Delta^{13}C_{CO2}$. The $\%^{13}C$ in CO_2 and in CH_4 was 24% and 51%, respectively, which was accounted for. Also the duration of the $^{13}CO_2$ incubation was 7 days compared to the $^{13}CH_4$ incubation of 9 days for which was corrected as well.

$^{13}CH_4$ incorporation into chlorophyll

Analyses of methane-derived ^{13}C incorporation into chlorophyll-*a* was performed with *S. cuspidatum* collected from the Mariapeel, the Netherlands. *Sphagnum* mosses were washed with demineralised water and incubated in 250 ml serum bottles. In each bottle, seven *S. cuspidatum* plants were incubated. Subsequently, 130 µmol of 99% ^{13}C-labelled methane was added after which the bottles were incubated in the light. In the control treatment the same amount of ^{12}C-labelled methane was added. After two weeks chlorophyll-*a* was extracted and purified using thin layer chromatography (TLC, on silicagel-60 Merck). Chlorophyll-a spots were scraped from the silicagel and eluted with ethanol. Isotope fractions of chlorophyll-*a* molecules were analysed with a Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass-spectrometer (type Bruker Biflex III, reflection mode) with alfa-cyano-5-hydroxy-cinnamic acid (CCA) as the matrix.

gDNA isolation

Sphagnum mosses were washed with sterile demineralised water and stored at -20ºC. Frozen mosses were grinded to powder in a mortar with liquid nitrogen, of which 5 ml were transferred to a 50 ml tube. PBS buffer was added and SDS and NaCl were added to a final concentration of 2% and 1M, respectively. One volume of phenol : chloroform : isoamylalcohol (25:24:1) was added and incubated at 65ºC for 2 h. After centrifugation for 20 min at 4000 rpm, the water phase was transferred into a new vial and combined with 1 volume chloroform:isoamylalcohol (24:1), after which it was mixed and centrifuged for 20 min at 4000 rpm. From the aqueous phase was, genomic DNA precipitated after adding 0.1 volume 3 M NaAc (pH 4.8) and 2 volumes of ethanol (100%). After incubation for 30 min at -20°C, the mixture was centrifuged for 20 min at 4000 rpm. The pellet was washed with 70% EtOH and dissolved in 0.5 ml MQ. The gDNA was purified by incubating the DNA with RNase for 15 min 37°C. Humic acids were removed by adding 500 µl Sephaglass bead suspension of the FlexiPrep Kit (Pharmacia P-L Biochemicals Inc.) and incubated for 1 min. Glass beads with bound DNA were washed twice with buffer and finally with 70% ethanol. The pellet was dried and DNA was dissolved in MQ. gDNA was stored at 4°C.

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Microarray

*pmo*A based microarray was performed as described previously[8], except the *pmo*A based PCR. Genomic DNA extracted from *Sphagnum* mosses was used as template in a touchdown PCR with the *pmo*A primer set a189/T7-A682. The PCR programme: 94°C for 5 min, 11 cycles of 1 min 94°C, 1 min 62°C with decrease of 1°C every cycle, 1 min 72°C, followed by 25 cycles of 1 min 94°C, 1 min 52°C, 1 min 72, followed by 10 min 72°C. This PCR product was diluted 100 times and used for a nested *pmo*A touchdown PCR with the A189/T7-mb661 following programme: 94 °C for 5 min, 11 cycles of 1 min 94 °C, 1 min 62°C with decrease of 1 °C every cycle, 1 min 72°C, followed by 14 cycles of 1 min 94 °C, 1 min 52°C, 1 min 72, followed by 10 min 72°C.

*mmo*X PCR

The *mmo*X based PCRs were performed using the primer sets mmoX1-mmoX2 (touchdown PCR 70-60 °C)[28], f882-r1403[29]and A-B[30], also called f166-r1401 and the combinations f882-B and A-r1403 (all touchdown PCR 63-53 °C). The same PCR program as described for the first PCR for the microarray has been used.

Supplementary Table S1 | Overview of sampling locations of collected *Sphagnum* mosses.

Country	Sample site	Coordinates	Sampling time	Sample name
Netherlands	Mariapeel	51° 24’ 90"N; 5° 54’ 90"E	December 2006, January 2007, May 2008	Mariapeel (MP)
	Hatertse Vennen	51° 47’ 4"N; 5° 48’ 2"E	May 2007, May 2008	Hatertse Vennen (HV)
UK	Bodmin moor	50° 27' 59"N; 4° 43' 32"W	January 2008	England
Russia	Northern Siberia	70° 37'N; 147° 53'E	July 2008	Northern Siberia
	Western siberia	60° 53' 26"N; 68° 41' 20"E	July 2008	Western Siberia
Canada	La mer bleue	45° 22'N; 75° 30'W	October 2008	Canada
Germany	Hespermoor	52° 25' 60”N; 7° 10' 60"E	September 2008	Germany
Sweden	Abisko, Stordalen	68° 20'N, 19° 00'E	October 2008	Sweden
Argentina	Av peat	54° 48′S 68° 18′W	December 2007, February 2008, April 2008	Tierra del fuego

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Supplementary Table S2 | Probes used for microarray analysis. Column numbers correspond to the order in which the probes are arranged on the microarray analysis shown in Figure 3.

Number	Name	Intended specificity
1	MbA557	*Methylobacter*
2	MbA486	*Methylobacter*
3	Mb460	*Methylobacter*
4	Mb_LW12-211	*Methylobacter*
5	Mb_SL#3-300	*Methylobacter*
6	Mb_SL299	soda lake *Methylobacter* isolates and clones
7	Mb_SL#1-418	soda lake *Methylobacter* isolates and clones
8	MmbB284	*Mmb. Buryatense - same region as Jpn284, but 3 MM vs. that one Methylobacter and Japanese strain related*
9	Jpn284	clone Jpn 07061
10	BB51-302	*Methylobacter*
11	Mb267	*Methylobacter*
12	Mb292	*Methylobacter*
13	Mb282	*Methylobacter*
14	Mb_URC278	*Methylobacter*
15	511-436	*Methylobacter*
16	LP10-424	*Methylobacter* LP 10 group
17	LF1a-456	*Methylobacter* LF 1a group
18	Mb_C11-403	*Methylobacter*
19	Mb380	*M.bacter broad group A universal?*
20	Mb271	*Methylobacter*
21	S14m2-270	*Marine type Ia cluster, S14m#2*
22	S14m2-406	*Marine type Ia cluster, S14m#2*
23	PS80-291	clone PS-80
24	MS1-440	*Marine type Ia cluster, Marine sediment #1*
25	Mm_pel467	*Methylomicrobium pelagicum*
26	Kuro18-205	*Marine type Ia cluster, Kuro18*
27	DS1-401	Deep sea cluster #1
28	Mm531	*Methylomonas*
29	Mm_M430	*Methylomonas*
30	Mm_RS311	*Mm.methanica, RS clade(10-286)*
31	Mm_ES294	*Methylomonas*
32	Mm_ES543	*Methylomonas*
33	Mm_ES546	*Methylomonas*
34	Mm_MV421	*Methylomonas*
35	Mm451	*Methylomonas*
36	Mm275	*Methylomonas*
37	Alp7-441	*Alpine soil Methylomonas, Alp#7 (10-282)*
38	peat_1_3-287	*Mehtylomonas*-related peat clones
39	Est514	*Methylomicrobium*-related clones
40	Mmb259	*Methylomicrobium album* + Landfill *M.microbia*
41	Mmb303	*Methylomicrobium album*
42	Mmb304	*Methylomicrobium album* + Landfill *M.microbia* and related

43	LW14-639	*Methylomicrobium LW14 group*
44	Mmb_RS2-443	*Methylomicrobium, Mmb_RS2*
45	Mmb562	*Mmb. album* and *Methylosarcina*
46	Mm229	*Deep-branching M.monas (?) group (WHmb3 related group)*
47	MsQ290	*M.sarcina quisquilliarum related*
48	MsQ295	*M.sarcina quisquilliarum*
49	LP20-644	*Methylomicrobium*-related clones
50	LP20-607	*LP20 group (Type Ia, deep branching-Mmb?)*
51	Ia193	Type I a (*M.bacter-M.monas-M.microbium)*
52	Ia575	Type I a (*M.bacter-M.monas-M.microbium-M.sarcina)*
53	Bsed516	*Marine sediment #2, Bsed*
54	SWI1-375	*Marine sediment #2, SW#1*
55	SWI1-377	*Marine sediment #2, SW#1*
56	Nc_oce426	*Nitrosococcus oceani*
57	DS2-287	*Deep sea #2, subgroup (N.coccus and Deep sea Type Ia 10-298)*
58	AIMS1-442	*Deep sea #2, AIMS#1*
59	DS2-220	*Deep sea #2, subgroup*
60	DS2-626	*Deep sea #2, subgroup*
61	USCG-225	Upland soil cluster Gamma
62	USCG-225b	Upland soil cluster Gamma
63	JR2-409	JR cluster #2 (California upland grassland soil)
64	JR2-468	JR cluster #2 (California upland grassland soil)
65	JR3-505	JR cluster #3 (California upland grassland soil)
66	JR3-593	JR cluster #3 (California upland grassland soil)
67	501-375	*Methylococcus*- related marine and freshwater sediment clones
68	501-286	*Methylococcus*- related marine and freshwater sediment clones
69	USC3-305	Upland soil cluster #3
70	Mc396	*Methylococcus*
71	MclT272	*Methylocaldum tepidum*
72	MclG281	*Methylocaldum gracile*
73	MclS402	*Methylocaldum szegediense*
74	MclS394	*Methylocaldum szegediense* and related
75	MclS400	*Methylocaldum szegediense* and related
76	MclE302	*Methylocaldum* E10
77	Mcl404	*Mc.capsulatus-Mcl.tepidum-Mcl. Gracile-Mcl.Szeg* and related
78	Mcl408	*Methylocaldum*
79	fw1-286	fw-1 group: *M.coccus-M.caldum* related marine and freshwater sediment clones
80	fw1-639	fw-1 group: *M.coccus-M.caldum* related marine and freshwater sediment clones
81	fw1-641	fw-1 group: *M.coccus-M.caldum* related marine and freshwater sediment clones
82	JHTY1-267	*JH-TY#1*
83	JRC4-432	Japanese rice cluster #4
84	OSC220	Finnish organic soil clones and related
85	OSC300	Finnish organic soil clones and related
86	JRC3-535	Japanese Rice Cluster #3
87	LK580	fw-1 group + Lake Konstanz sediment cluster
88	RSM1-419	*RSM#1*

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89	JHTY2-562	*JH-TY#2*
90	JHTY2-578	*JH-TY#2*
91	JRC2-447	Japanese Rice Cluster #2
92	LW21-374	LW21 group
93	LW21-391	LW21 group
94	M90-574	*M.coccus-M.caldum* related marine and freshwater sediment clones
95	M90-253	*M.coccus-M.caldum* related marine and freshwater sediment clones
96	Mth413	*Methylothermus*
97	Mha-500	*Methylohalobius - M.thermus and related ?*
98	DS3-446	Deep sea cluster #3
99	PmoC640	*PmoC*
100	PmoC308	*PmoC*
101	Ib453	Type I b (*M.thermus-M.coccus-M.caldum* and related)
102	Ib559	Type I b (*M.thermus-M.coccus-M.caldum* and related)
103	McyB304	*M.cystis B (parvus/echinoides/*strain M)
104	Mcy255	*M.cystis B (parvus/echinoides/*strain M)
105	Mcy459	*Methylocystis*
106	Mcy264	*Methylocystis*
107	Mcy270	*Methylocystis*
108	Mcy413	*Methylocystis*
109	Mcy522	*Methlocystis* A + peat clones
110	Mcy233	*Methylocystis*
111	McyM309	*M.cystis strain M and related*
112	Peat264	peat clones
113	MsS314	*Methylosinus sporium*
114	MsS475	*Methylosinus sporium*
115	Msi263	*Methylosinus sporium* + 1 *Msi.trichosporium subclaster*
116	Msi423	*Methylosinus*
117	MsT214	*Methylosinus trichosporium* OB3b and rel.
118	Msi520	*Methylosinus trichosporium*
119	Msi269	*Methylosinus trichosporium*
120	Msi294	*Methylosinus*
121	ARC2-518	*Deep branching type II clade ARC2 - Methylosinus trichosporium 15-084 group?*
122	Msi232	*M.sinus*+ most *M.cystis* -considered as additional type II probe
123	II509	Type II
124	II630	Type II
125	Alp8-468	*Type II novel pmoA, Alpine cluster Alp#8*
126	xb6-539	Novel *pmoA* copy of type II and related environmental clones
127	LP21-190	Novel *pmoA* copy of type II and related environmental clones
128	LP21-260	Novel *pmoA* copy of type II and related environmental clones
129	NMcy1-247	Novel *pmoA* copy of *M.cystis #1* (?)
130	NMsi1-469	Novel *pmoA* copy of *M.sinus*
131	NMcy2-262	Novel *pmoA* copy of *M.cystis #2* (?)
132	LP21-436	*Mcy + Msi novel pmoA #1 groups*
133	NMsiT-271	Novel *pmoA* copy of *M.sinus trichpsporium* (?)
134	LP21-232	Novel *pmoA* copy of type II and related environmental clones
135	RA14-299	RA14 related clones
136	RA14-594	RA14 related clones

137	RA14-591	RA14 related clones
138	Wsh1-566	Watershed + flodded upland cluster 1
139	Wsh2-491	Watershed + flodded upland cluster 2
140	Wsh2-450	Watershed + flodded upland cluster 2
141	B2rel251	*Methylocapsa*-related clones
142	B2-400	*Methylocapsa*
143	B2-261	*Methylocapsa*
144	B2all343	*Methylocapsa* and related clones
145	B2all341	*Methylocapsa* and related clones
146	pmoAMO3-400	clone pmoA-MO3
147	pmoAMO3-486	*MO3 group*
148	pmoAMO3-511	*MO3 group*
149	Ver330	*Verrucomicrobia, all pmoA1+pmoA2*
150	Ver307	*Verrucomicrobia, all pmoA2*
151	Ver285	*Verrucomicrobia, Ma.fum pmoA2+Ma.kam. pmoA2*
152	Ma_F1-594	*Ma.fum. pmoA1*
153	Ma_I1-312	*Ma.inf. pmoA1*
154	Ma_I1-401	*Ma.inf. pmoA1*
155	Ma_F3-638	*Ma.fum. pmoA3*
156	Ma_F3-542	*Ma.fum. pmoA3*
157	ESR-579	ESR (Eastern Snake River) cluster
158	M84P22-514	environmental clones of uncertain identity
159	TUSC409	Tropical Upland Soil Cluster #2
160	TUSC502	Tropical Upland Soil Cluster #2
161	mtrof173	Universal
162	mtrof362-I	Methanotrophs
163	mtrof661	Methanotrophs
164	mtrof662-I	Methanotrophs
165	mtrof656	Methanotrophs

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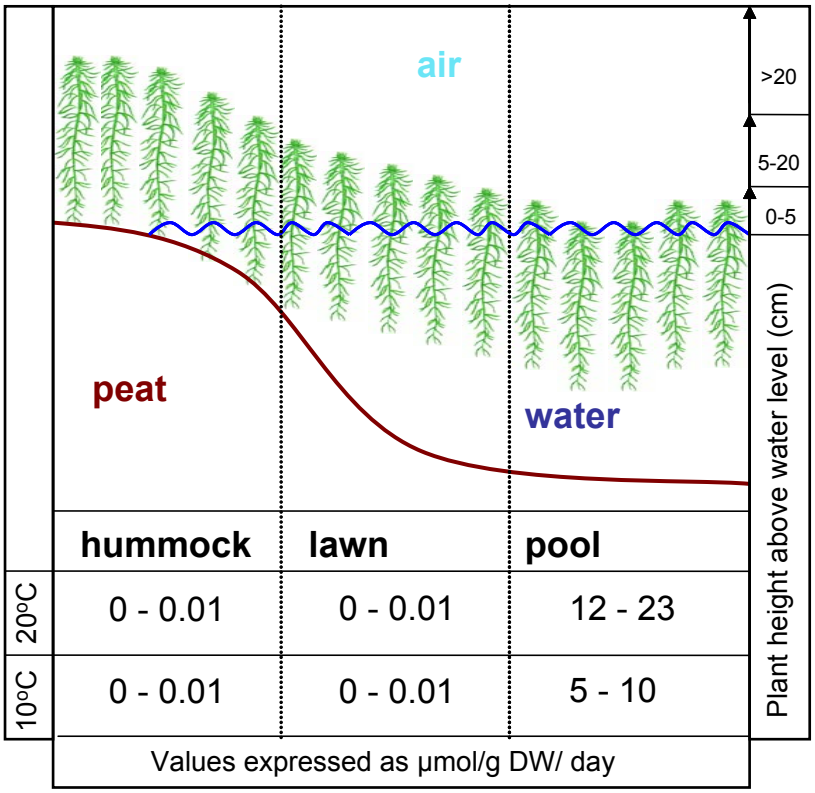
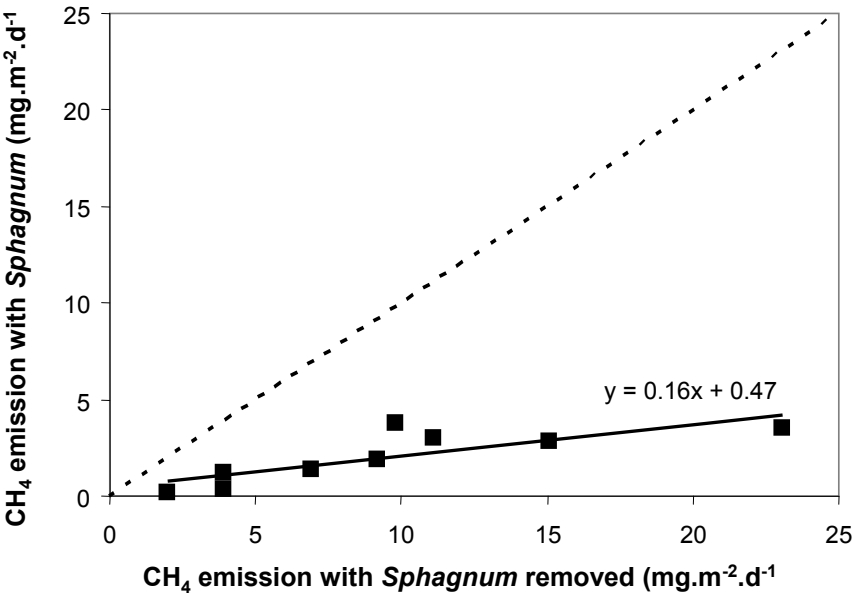


Supplementary Figure S1 | Methane oxidation rates at different temperatures. Schematic representation of the three different tested vegetation types, all *Sphagnum magellanicum,* with the average methane oxidation rates (in µmol/gDW/day) found in Tierra del Fuego at two different temperatures. Values are means of at least 6 incubations ± S.D.



Supplementary Figure S2 | Comparison of methane emission from peat cores before and after removal of the *Sphagnum* layer. Emissions were determined from 9 different cores. Emission with *Sphagnum* was plotted against the emission after removal. The dotted line indicates the trend when no changes in methane oxidation rates would have occurred.

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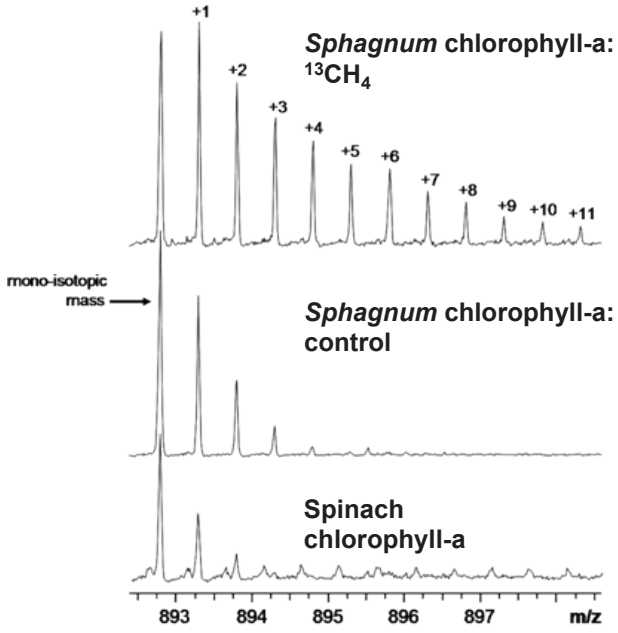

Supplementary Figure S3 | ^{13}C enrichment of chlorophyll-*a* extracted from *S. cuspidatum*. MALDI-TOF spectra of chlorophyll-a extracted from *S. cuspidatum* (Mariapeel, The Netherlands) incubated with 99% ^{13}C-labelled methane (top), compared to natural ^{13}C abundance in *Sphagnum* (middle) and spinach (bottom). The spectra show the relative abundance of chlorophyll-*a* with a mono-isotopic mass (100% ^{12}C) and chlorophyll-a containing one ^{13}C, two ^{13}C, three ^{13}C etc. consecutively.